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Biocontrol efficacy of *Trichoderma* spp. against wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*

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ABSTRACT

The objective of this paper was to evaluate the efficacy of the native isolates of *Trichoderma* species to promote the growth and yield parameters of tomato and to manage *Fusarium* wilt disease under *in vitro* and *in vivo* conditions. The dominant pathogen, which causes *Fusarium* wilt of tomato, was isolated and identified as *Fusarium oxysporum* f. sp. *lycopersici* (FOL). Fifteen native *Trichoderma* antagonists were isolated from healthy tomato rhizosphere soil in different geographical regions. Under *in vitro* conditions, the results revealed that *Trichoderma harzianum* (ANR-1) isolate was found to effectively inhibit the radial mycelial growth of the pathogen (by 53%) when compared to all other isolates. Under greenhouse conditions, the application of *Trichoderma harzianum* (ANR-1) exhibited the least disease incidence (by 15.33%). Also tomato plants treated with *Trichoderma harzianum* (ANR-1) isolate showed a significant stimulatory effect on plant height (by 73.62 cm) and increased the dry weight (by 288.38 g) of tomato plants in comparison to other isolates and untreated control.

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop grown in almost all parts of India. Its popularity is due to its high nutritive value, diversified use, and nutritional significance as a source of vitamins A and C. It occupies number one position in its nutrient contribution to human diet. In Tamil Nadu, tomato is grown in an area of 22,433 ha, with a production of 2,82,912 tonnes and a productivity of 12,611 kg/ha [1]. It is affected by several diseases, reflecting negatively on plant growth and the produced yield.

Out of these, pathogenic fungi especially, the wilt caused by species of *Fusarium* remain to be a challenging task in terms of management [2, 3]. Wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen is one of the most economically important diseases world-wide [3, 4]. As *Fusarium* wilt is soil-borne in nature, application of fungicides to control this disease is not practical. Besides, chemicals pose serious health hazards to an applicator as well as to a consumer of the treated material.

In addition to target organism, pesticides also kill various beneficial organisms. Their toxic forms persist in soil and contaminate the whole environment [5]. Prospects of biological control of soil-borne plant pathogens using most promising bio-control agent, the genus *Trichoderma* has been described [6, 7]. Successful reductions of *Fusarium* wilt in many crops with application of different species of *Trichoderma* have been found [8, 9, 10]. However, it is also reported that all the isolates of *Trichoderma* spp. are not equally effective in control of pathogen *in vitro* [11, 12] and *in vivo* conditions to control diseases. Therefore, specific isolates are needed for successful control of a particular pathogen.

Therefore the objectives of the present study were to assess the ability of fifteen isolates of *Trichoderma* species in suppressing the populations of FOL in tomato under *in vitro* and *in vivo* conditions.

2. MATERIALS AND METHODS

2.1. Isolation and purification of pathogens

Infected vascular tissues from stem and root regions of tomato cultivar (PKM 1) showing wilt symptoms were collected separately from farmer's field. Tissue bits were surface sterilized with 10 per cent sodium hypochlorite for 5-10 min. and subsequently three washings with sterile distilled water.

Then, they were placed on potato dextrose agar (PDA) medium separately and incubated at the laboratory conditions at 25 ± 3^{o} C for five days (Fig.1).

The fungi were purified separately by transferring the tip of the mycelia into PDA slants and maintained as stock cultures for further studies (Fig.2).

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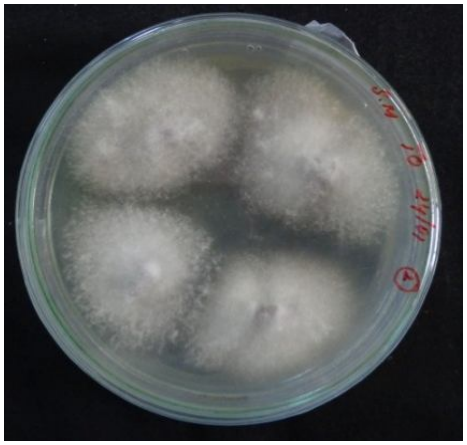


Fig. 1: Isolation of FOL from wilt infected tomato tissue bits.

Fig. 2: Axenic culture of FOL.

2.2. Isolation and maintenance of fungal native antagonists from tomato rhizosphere soil

Rhizosphere soil from healthy tomato plants were collected from different locations. The identified *Trichoderma* antagonists *viz*., *T. hamatum*, *T. harzianum*, *T. koningi*, *T. longiconis* and *T. viride* were isolated by serial dilution technique using *Trichoderma* selective medium (TSM) and compared with the isolate maintained in the laboratory [13].

2.3. *In vitro* effect of *Trichoderma* antagonists against FOL pathogen

Dual culture technique as described earlier was followed. Nine mm disc of fifteen days old fungal cultures were placed on PDA medium one cm away from the edge of the plate, separately. *Trichoderma* spp. (9 mm disc) was placed at opposite side of the Petri plate. Three replicated plates for each treatment was maintained and incubated at 25±3°C. Per cent inhibition over control was calculated [14] as per the formulae

$$PI = \frac{C - T}{C} \times 100$$

Where,

PI = Per cent inhibition over control

C = Growth of test pathogen with absence of antagonist (mm)

T = Growth of test pathogen with antagonist (mm)

2.4. Development of talc based formulation of *Trichoderma* spp.

The talc based formulation of *Trichoderma* spp was prepared according to the method described by [15]. Nine mm disc of *T. viride* was inoculated into 100 ml molasses yeast medium and incubated at room temperature (28± 2°C) for 5 days. The mycelial mat was mixed with talc powder in 1:2 ratio and shade dried. To this, carboxy methyl cellulose was added at the rate of 0.5 percent as sticker. The product was shade dried to 20 per cent and packed in polypropylene bags and sealed.

2.5. Greenhouse experiment:

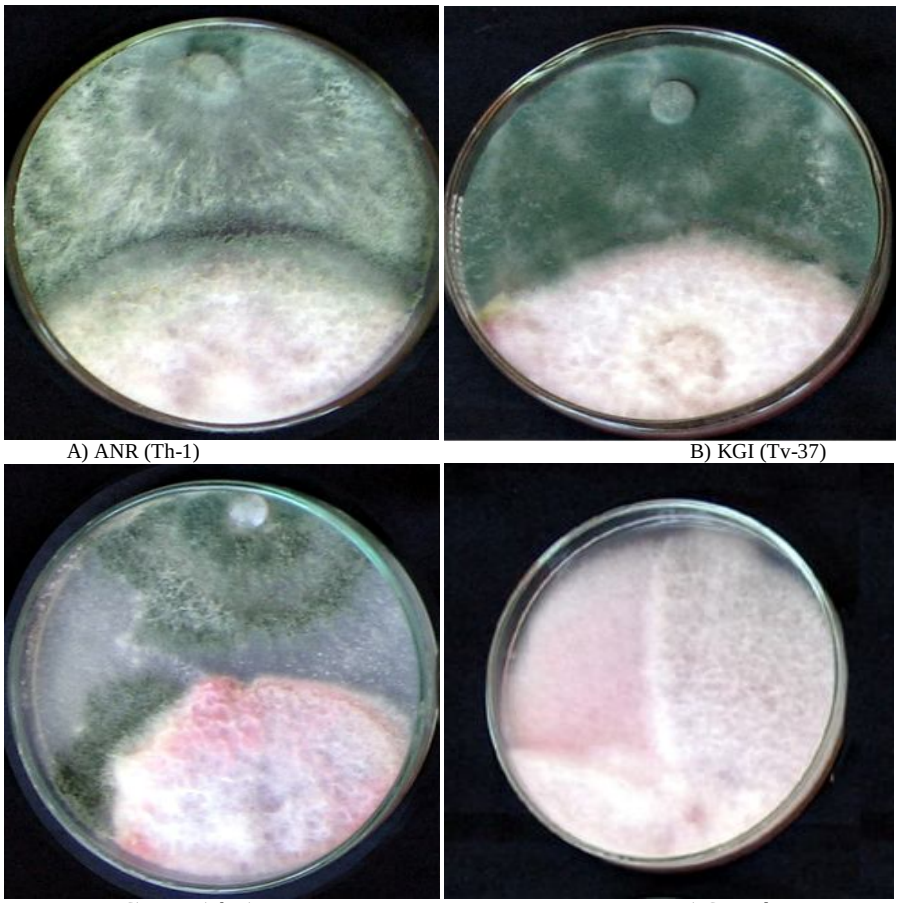
A pot culture study was conducted to test the antagonistic potential of *Trichoderma* spp. against *F. oxysporum* f. sp. *lycopersici*. Potting mixture (red soil: sand: decomposed FYM at 1:1:1 w/w/w) was prepared and autoclaved 1 hr for two consecutive days and filled in earthern pots of 5 kg capacity. Tomato (var.PKM1) seeds were sown in autoclaved pot mixture in earthern pots. After 25 days, the seedlings were pulled out from the pots and dipped in their respective formulation for 2 h ensuring that the roots alone were immersed in the inoculum and then transplanted in pots at the rate of four seedlings per pot (5 kg capacity). ANR-1, KGI -3, RTM-5, KPI-9 and EPI-4 isolates were effective against *F. oxysporum* f. sp. *lycopersici* under *in vitro* were selected. Soil drenching with the formulation was done 15 days and 30 days after transplantation. The wilt pathogen *F. oxysporum* f.sp. *lycopersici* mass multiplied on sand maize medium was incorporated in to the pots at 5 per cent (w/w). The observation on the per cent disease incidence was recorded at the time of harvest. Each treatment was replicated thrice in Completely Randomized Block Design (CRD).

The treatment details were as follows;

Trt. No	Designation of *Trichoderma* Native isolates	Treatment details
T1	KPI-9	Seedling dip @ 0.2 % + Soil application at 15 and 30 DAT @ 0.2 %
T2	KGI -3	Seedling dip @ 0.2 % + Soil application at 15 and 30 DAT @ 0.2 %
T3	ANR-1	Seedling dip @ 0.2 % + Soil application at 15 and 30 DAT @ 0.2 %
T4	RTM-5	Seedling dip @ 0.2 % + Soil application at 15 and 30 DAT @ 0.2 %
T5	EPI-4	Seedling dip @ 0.2 % + Soil application at 15 and 30 DAT @ 0.2 %
T6	Carbendazim (0.1%)	Seedling dip @ 0.2 % + Soil drenching at 15 and 30 DAT @ 0.2 %
T7	Healthy control (without pathogen)	
T8	Inoculated control (with pathogen)	

2.6. Statistical analysis

The data were statistically analyzed [16] and the treatment means were compared by Duncan's Multiple Range Test (DMRT). The package used for analysis was IRRI-Stat version92-a developed by International Rice Research Institute Biometrics Units, The Philippines.



A) ANR (Th-1) B) KGI (Tv-37)

C) RTM (Th-5) D) Control

Fig. 3: Antagonistic efficacy of *Trichoderma* spp. against tomato wilt pathogen (FOL) under *in vitro* condition.

Table. 1: Fungal native antagonists isolated from rhizosphere soil of tomato.

Sl. No.	Place of collection	Colony color	Fungal native antagonists
1	Annamalai Nagar	Dark green	*Trichoderma harzianum*
2	Annamalai Nagar	Dark green	*Trichoderma viride*
3	Ramanathapuram	Dark green	*Trichoderma hamatum*
4	Ramanathapuram	Dark green	*Trichoderma* sp.
5	Krishnagiri	Dark green	*Trichoderma* sp.
6	Krishnagiri	Dark green	*Trichoderma viride*
7	Dharmapuri	Dark green	*Trichoderma longibrachiatum*
8	Dharmapuri	Green	*Trichoderma* sp.
9	Edappadi	Dark green	*Trichoderma* sp.
10	Edappadi	Dark green	*Trichoderma* sp.
11	Oddanchatram	Dark green	*Trichoderma* sp.
12	Oddanchatram	green	*Trichoderma viride*
13	Kovilpatti	Dark green	*Trichoderma hamatum*
14	Kovilpatti	Dark green	*Trichoderma* sp.
15	Kovilpatti	Dark green	*Trichoderma longibrachiatum*

Table. 2: Promising fungal native antagonists.

Name of agro climatic zone	Fungal Native Antagonists	Designation of *Trichoderma* isolates	Colony color
Annamalai Nagar	*Trichoderma harzianum* -1	ANR -1	Dark green
Annamalai Nagar	*Trichoderma* sp-4	ANR -4	Green
Ramanathapuram	*Trichoderma hamatum-5*	RTM-5	Green
Ramanathapuram	*Trichoderma sp-7*	RTM-7	White
Krishnagiri	*Trichoderma viride-3*	KGI-3	Dark green
Kovilpatti	*Trichoderma longibrachiatum-9*	KPI -9	Dark green
Edappadi	*Trichoderma* sp.	EPI-4	Whitish green
Edappadi	*Trichoderma* sp.	EPI-8	Green

Table. 3: Effect of *Trichoderma* antagonists on the mycelia growth of *F. oxysporum* f. sp. *lycopersici* under *in vitro* conditions.

Trt. No.	Fungal Native Antagonists	*Mycelial growth (mm)	Percent inhibition over control
T1	RTM-5	62.00^{c}	31.11^{c}
T2	EPI-4	67.10^{d}	25.44^{d}
T3	RTM-7	77.60^{e}	11.53^{e}
T4	EPI-8	78.60^{e}	12.67^{e}
T5	KPI-9	65.50^{cd}	27.22^{cd}
T6	KGI -3	55.70^{b}	38.12^{b}
T7	ANR-1	42.30^{a}	53.00^{a}
T8	RTM-12	88.50^{f}	1.66^{f}
T9	ANR-4	78.60^{e}	12.67^{e}
T10	Control	90.00^{f}	0.00

*Mean of three replications

In a column, mean followed by a common letter are not significantly different at the 5% level by DMRT.

Table. 4: Efficacy of *Trichoderma* bioformulation in the management of fusarial wilts of tomato cv. PKM1 under greenhouse conditions.

Trt.No	Fungal Native Antagonists	FOL	*Plant height (cm)	*Per cent disease incidence	*Fruit yield g/plant
T1	KPI-9	+	61.76^{e}	25.50^{e}	186.78^{d}
T2	KGI -3	+	67.00^{b}	17.45^{c}	249.87^{b}
T3	ANR-1	+	73.62^{a}	15.33^{b}	288.38^{a}
T4	RTM-5	+	65.91^{c}	22.50^{d}	228.43^{c}
T5	EPI-4	+	63.13^{d}	25.10^{e}	185.97^{d}
T6	Carbendazim (0.1 %)	+	63.27^{d}	9.10^{a}	227.23^{c}
T7	Inoculated control	+	57.73^{f}	57.75^{f}	110.73^{e}
T8	Healthy control	-	60.98^{e}	25.80^{e}	187.86^{d}

+/- (Presence/Absence of wilt pathogen)

*Mean of three replications

In a column, mean followed by a common letter are not significantly different at the 5% level by DMRT.

3. RESULTS

3.1. *In vitro* screening of bacterial native antagonists against the radial mycelial growth *F. oxysporum f. sp. lycopersici*

Fifteen native isolates of *Trichoderma* spp. were screened for their *in vitro* antagonism against the *F. oxysporum* f. sp. *lycopersici* by dual cultural technique. The results indicated that ANR-1 inhibited the mycelial growth of *F. oxysporum* f. sp. *lycopersici* to an extent of 53.00 per cent over control (Fig.3). This was followed by KGI-3 (38.12 %), RTM-5 6 (31.11%) and KPI-9 (27.22 %) (Table 3).

3.2. Effectiveness of native *Trichoderma* antagonists on wilt incidence and yield parameters under glasshouse conditions

The application of *Trichoderma* native antagonists through seedling dip and soil application was found effective in suppressing wilt incidence (by 15.33-25.50%). Conspicuously, an application of ANR-1 antagonistic fungal formulation was recorded least wilt incidence (by 15.33%) followed by KGI-3 (by 17.45 %) compared to other isolates (Table 24). Among the treatments, Carbendazim (0.1%) was found to be the most effective and recorded the least wilt incidence of 9.10 % compared to control (57.75%). Also the results of this experiment revealed that the application of ANR-1 antagonistic fungal formulation significantly increased the plant height (by 73.62 cm) and fruit yield (by 288. 38 g) when compared to other isolates and untreated control (Table 4).

4. DISCUSSION

Fungal species belonging to the genus *Trichoderma* are worldwide in occurrence and easily isolated from the soil. The potential of *Trichoderma* species as bioconrol agents against various plant diseases has been reported by several workers [17, 18]. In the present investigation, fungal antagonist ANR-1 isolate caused highly significant reduction in tomato wilt incidence under *in vitro* and *in vivo* conditions. The inhibitory effect of these bioagents against tested pathogen was probably due to competition and/or antibiosis.

Demands for *in vitro* effectiveness of *Trichoderma* against species of *Fusarium* have been reported [19]. The antagonist *Trichoderma harzianum*, *T. coningi* and *T. viride* were reported to be equally antagonistic to *F. udum* under *in vitro* [20]. [21] reported that *Trichoderma* spp. successfully controlled *Fusarium* spp. on cotton, wheat and muskmelon. Sesame seeds treated with three isolates of *T. viride* reduced the pre- and post-emergence damping off caused by *R. solani* and *F. oxysporum* f. sp. *sesami* under pot culture and field conditions.

In the present investigation, the plant height and fruit yield were also increased in ANR-1 treated plants. Similar results on increased plant growth due to application of *Trichoderma gamsii* in cereals and legume crops [22]. The increase in plant growth might be associated with secretion of auxins, gibberellins and cytokinins.

The increase in biomatter production may be due to the production of plant growth promoters or through indirect stimulation of nutrient uptake and by producing siderophore or antibiotics to protect plants from deleterious rhizosphere organisms. Therefore, the antagonist *T. harzianum* (ANR-1) is chosen to be the most promising bio-control agent for *F. oxysporum* f.sp. *lycopersici*. On the base of present study the bioagents of fungi, might be exploited for sustainable disease management programs to save environmental risk.

5. CONCLUSION

The present evaluation thus gave clear indication that the isolates of *T. harzianum* (ANR-1) and *T. viride* (KGI-3) isolated from tomato rhizosphere are strong and virulent antagonists, which can be effectively used in the management of tomato wilt. Combination of seedling dip and soil application appears to be most effective.

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